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This report presents data: On the evaluation of antimalarial drugs. Experimental drugs GJ-287 (WR282650; BP20546) and GJ-QZ (WR282651; BP20537) were non toxic when given to Aotus orally at 20 mg/kg for three consecutive days and only transient changes on blood leukograms and liver enzyme ALT were detected. These drugs were not effective alone or in combination with Chloroquine when Aotus monkeys infected with *Plasmodium falciparum* FVO strain were treated for three consecutive days. Significant differences on Lymphocytes and Granulocytes counts were found between male and female Aotus when hematological and renal chemistry values of feral laboratory adapted Aotus monkeys were determined.

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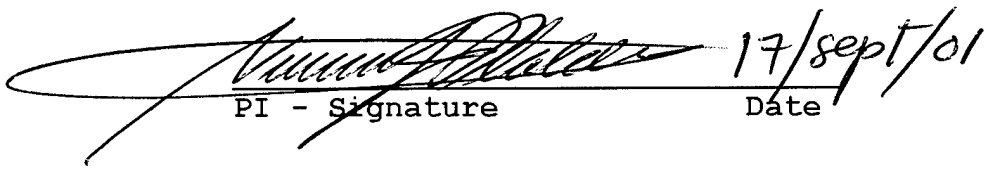

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INTRODUCTION:

Each year there are 300-500 million new infections and 2-5 million deaths attributable to malaria that occur primarily in countries in the tropics, particularly in sub-Saharan Africa **(4)**. During the past 10-20 years the malaria problem has intensified in some parts of the world because parasites have developed resistance to drugs used for treatment and prevention; the anopheles mosquito, which transmits the parasite to humans, has developed resistance to insecticides, and control efforts have been reduced as resources have diminished in some developing countries **(7)**.

The use of *Aotus lemurinus lemurinus* (Panamanian *Aotus* monkey), kariotypes VIII and IX **(16)** as a model to study malaria drug resistance and vaccine efficacy, have been ongoing at Gorgas Memorial Laboratory since 1976, due in part to the availability of this monkey in Panama **(20)**, and also to the increasing drug resistance exhibited by the highly pathogenic *Plasmodium falciparum* parasites in Asia, Africa, and Latin America, and more recently *Plasmodium vivax* in the Melanesian and Indonesian archipielago **(21)**. Previously, Schmidt **(26,27)** used the Colombian *Aotus* as the experimental host for antimalarial drug studies, but embargoes imposed by South American countries on the exportation of monkeys in the mid 1970's seriously restricted the use of *Aotus* for biomedical research in the United States, and in 1976 the project was transferred to Gorgas Memorial Laboratory where Panamanian *Aotus* were available for research. Five strains of *P. falciparum*, Vietnam Smith, Uganda Palo Alto , Vietnam Oak Knoll (FVO) , Santa Lucia **(5)**, and a C2A mefloquine resistant clone, and three strains of *P. vivax* Chesson (chloroquine sensitive), New Guinea AMRU-1 (chloroquine resistant) and Sal-1, have been adapted to Panamanian Aotus.

These strains exhibit diverse susceptibility and/or resistance to standard antimalarial agents. The course of untreated infections in Panamanian *Aotus* has been characterized and compared with that in *Aotus* of Colombia **(25)**. Overall, the virulence of these strains was less in Panamanian than in Colombian owl monkeys, as indicated by lower mortality rates of Panamanian monkeys during the first 30 days of patency. Maximum parasitemias of the Vietnam Smith and Uganda Palo Alto strains were, however, significantly higher during the first 15 days of patency in Panamanian than in Colombian owl monkeys. These quantitative differences in infection parameters between Panamanian and Colombian owl monkeys have not invalidated the use of the former for evaluation of new antimalarial drugs.

Numerous candidate antimalarial drugs of diverse chemical classes have been evaluated against trophozoite-induced infections of one or more *P. falciparum* strains during the course of these contracts. In seeking alternatives to primaquine, two 8-aminoquinolines proved to be active against the blood stages of *P. falciparum* **(2, 18)**. Desferrioxamine, an iron-specific- chelating agent, was shown to suppress parasitemias of the virulent

Uganda Palo Alto strain of *P. falciparum* **(23)**. The *in vitro* activity of two halogenated histidine analogs was not confirmed by evaluation against *P. falciparum* infections in owl monkeys **(22)**.

Chloroquine-resistance of *P. falciparum* represents the greatest challenge in developing effective antimalarial drugs. Reversal of chloroquine-resistance in *P. falciparum, in vitro*, was achieved by the co-administration of verapamil (a calcium channel blocker) plus chloroquine **(17)**. Other in vitro studies have shown that there is a significantly greater efflux of chloroquine from erythrocytes containing falciparum parasites resistant to chloroquine than from red cells parasitized by chloroquine-sensitive falciparum malaria **(14)**. Calcium channel blockers appear to prevent this active efflux of chloroquine, thus allowing the drug to accumulate to parasiticidal levels.

Based upon the success of *in vitro* reversal of chloroquine-resistance, trials were initiated to determine if resistance could be reversed in *Aotus* infected with the chloroquine-resistant Vietnam Smith strain of *P. falciparum*. Six calcium channel blockers, or similarly acting drugs, were co-administered with chloroquine in diverse regimens. The desideratum of chloroquine-resistance reversal was administration of a single course of treatment, with parasite clearance and infection cure. Suppression of parasitemia was obtained during an initial course of treatment, but parasite clearance and cure occurred in some instances only after re-treatment. Such infection parameters were similar to those in monkeys with self-limited infections and cure could be attributed to acquired immunity.

Limited trials with desipramine, Norpramin , a tricyclic psychotropic drug, demonstrated the feasibility of reversing chloroquine-resistance in vivo **(1)**. parasite clearance was obtained, but the infection was not cured.

Subsequently, in vivo reversal of chloroquine resistance was obtained with combinations of chloroquine plus chlorpromazine or prochlorperazine. Such reversal was exhibited by rapid suppression and clearance of parasitemia, resulting in infection cure without retreatment **(15)**.

Evaluation of two oil-soluble derivatives of artemisinin, artemether and arteether, demonstrates that both possess similar activity to cure infections of a multi-drug resistant *P. falciparum* strain in *Aotus* **(28)**.

Some strains of *P. vivax* from Melanesia and the Indonesian archipelago have demonstrated resistance to treatment with chloroquine **(19, 24)**. Unlike chloroquine-resistant falciparum malaria, there exists no easy alternative to chloroquine-resistant strains of vivax malaria. Using WR 238605 alone or in combination with chloroquine in Panamanian *Aotus* monkeys it was demonstrated that WR238605 is a an alternative treatment for chloroquine-resistant vivax malaria **(21)**. The compound WR 238605 is a primaquine analog developed by the US Army as a better tolerated, more effective replacement for primaquine. Recent studies done at Gorgas Institute with Artemisin derivative durgs developed by the U.S. Army such as Artelinic acid demonstrated its efficacy against the FVO strain of *P. falciparum* when administered orally to *Aotus l. lemurinus.*

Both the purpose and methods of approach of the present work remains essentially unchanged since 1976, viz to ascertain the antimalarial activity of drugs against *P. falciparum* and *P. vivax* in *Aotus*. The method of approach may vary on an ad hoc basis, such as administering a combination of drugs.

The long term goal of the second part of this project is to develop fully protective plasmid DNA vaccines that induce protective immune responses against the sporozoite, liver and erythrocytic stages of *P. falciparum*. If successful, it will establish, for the first time, that plasmid DNA vaccines can protect non-human primates, a critical step forward for the use of plasmid DNA vaccines in humans.

Vaccines are aimed at inducing immune responses that disrupt the complex cycle of the parasite at one more points: anti-sporozoite antibodies that prevent invasion of hepatocytes; cytotoxic T lymphocytes, cytokines, and antibodies that eliminate infected hepatocytes; antimerozoite antibodies that prevent invasion of erythrocytes; antibodies that neutralize parasite exoantigens known to induce harmful cytokine responses; antibodies that attack infected erythrocytes; cytokines that kill parasites within erythrocytes; and, anti-sexual stage antibodies that prevent the development of sporozoites in the mosquito.

Previous trials of malaria blood stage vaccines have shown that the Panamanian *Aotus**P. falciparum* model to be suitable for this purpose. **(8-10)**.

Immunogenicity studies of a plasmid DNA vaccines encoding the circumsporozite *P. yoelli* rodent malaria gene (PyCSP) in Panamanian *Aotus* monkeys demonstrated that the intradermal route of inoculation (ID) induces a higher level of antibodies than the intramuscular route (IM). Antibody levels induced in this manner reached a peak at week 9 and titers declined to 50% their peak value by week 14. When boosted at week 46 antibody levels increase 4 fold by week 49. This was comparable to antibodies generated with a Multiple Antigen synthetic peptide vaccine (MAP) delivered with an adjuvant **(4)**

We have used this inmunization scheduled to test single or multi-gene DNA plasmid vaccines in *Aotus* monkeys. Additionally we have tested the ability of recombinant cytokines to enhance the immunogenicity and protective efficacy of the DNA vaccines. Preliminary using a small group of *Aotus l. lemurinus* (n = 3) demonstrated partial, but incomplete, protection with a DNA vaccines for either AMA-1 or EBA-175 alone. These studies indicated that animals which received the vaccine candidates, had a short, but apparent significant delay in the onset of parasitemia {approximately 33% (1 of 3) self-cured, whereas none of the control animals did}. However, since the number of animals per group in each of these pilot studies were small, it was not possible to determined the absolute efficacy of these candidate vaccines, but these experiments suggested to the

investigators that further studies were warranted. MSP-1, when used as a protein/peptide vaccine formulation, provided protection from a *P. faciparum* infection in *Aotus* monkeys and we have demonstrated that, in mice and in Rhesus monkeys, the cytokine GM-CSF augmented both immunogenicity of a malaria DNA vaccine (personal communication. W. Weiss). We have now completed a pilot experiment to determine if *Aotus* Granulocyte-Macrofage-Colony Stimulating Factor (aGM-CSF) can augment immunogenicity and protective efficacy of a multi-gene erythrocytic vaccine.

In addition, synthetic oligodeoxynucleotides containing CpG motifs enhance immunogenicity of a peptide malaria vaccine when tested in Panamanian Aotus **(11)**. Different vaccine formulations, routes and methods of administration with a comparable Hepatitis B Plasmid DNA vaccine were explored in Panamanian Aotus in order to elucidate the best route and methods of immunization for a plasmid DNA malaria vaccine **(6)**. Further studies with single or multistage antigen plasmid DNA vaccines have been conducted or are in progress in Panamanian Aotus with variable results. Herein, we report partial protection obtained in Aotus monkeys immunized with either plasmid or recombinant protein in a primary and boosting immunization schedule using $MSP1_{42}$ as an antigen.

We have also tested the effect of prior *P. falciparum* infection on the immunogenicity of a DNA vaccine, obtaining partial protection in 67% of the monkeys **(12)**. Also, evaluated in Aotus monkeys the characteristics of *P. falciparum*-induced anemia in two different experimental settings and hypothesis that a non-antibody/non-complement-mediated lysis of uninfected erythrocytes was the principal cause of anemia, and that bone marrow suppression and lysis of infected erythrocytes contributed to the anemia **(13)**. In addition, we tested the hypothesis that *a P. falciparum* ligand, EBA-175 region II (RII), can be used as an immunogen in Aotus to induce antibodies that block the binding of RII to erythrocytes and thus inhibit parasite invasion of erythrocytes **(29)**.

The purpose of this report is to: 1) Present data on the evaluation of potential antimalarial activity of drugs in the pre-clinical model of *Aotus l. lemurinus* (Panamanian night monkey) experimentally infected with *P. falciparum* or *P. vivax*, and 2) data on plasmid DNA and recombinant protein malaria vaccine experiments. These studies were supported by the U.S. Army and the U.S. Navy Malaria Programs.

BODY:

I. Experimental Methods

The first aim of this project is to evaluate the potential antimalarial activity of drugs, or combination thereof, in the preclinical model of *Aotus*

experimentally infected with *P. falciparum* (or *P. vivax*). Specifically, the vertebrate host is *A. l. lemurinus*, the Panamanian night monkey. These animals are either feral, laboratory adapted or laboratory born. No naturally acquired, human plasmodium infection has been reported in *Aotus*. The Vietnam Smith/RE strain of *P. falciparum* was adapted to *Aotus* of Colombian origin in 1971 **(26)** and in Panamanian *Aotus* in 1976. **(25)**. The course of untreated infections, essential for comparison with treated infections, has been documented in Panamanian *Aotus* **(25)**. This plasmodium strain is resistant to maximally tolerated doses of chloroquine, pyrimethamine, and quinine **(27)**.

To initiate an experiment, infected blood (with 2.5% sodium citrate as the anticoagulant) from an untreated *Aotus* was diluted appropriately in chilled saline (0.85%) or RPMI, such that each milliliter contained 5,000,000 parasites. This amount was inoculated into the saphenous vein of experimental and control monkeys.

Blood films, prepared and examined daily beginning on the first post-inoculation day, were stained with Giemsa. Parasitemias were evaluated as follows: negative, if no parasites were detected on a thick blood film after examination for at least 5 minutes; <10 parasites per cmm, if positive only on the thick blood film; parasite enumeration was by the Earle-Perez method and reported as the number of parasites per cmm. **(3)**

Blood films from untreated *Aotus*, serving as passage and/or control subjects, were prepared and examined daily during the primary patent period, and daily thereafter for at least three consecutive days after parasites could last be detected on thick blood films. When parasitemia had cleared, films were made and examined twice weekly until a total of 100 negative days had been recorded. If recrudescence occurred, blood films were obtained again on a daily basis.

Parasitemias were evaluated daily during the treatment period and until blood films were negative for at least seven consecutive days. The frequency of smearing was then reduced to two times per week (Monday and Thursdays or Tuesdays and Fridays). If no recrudescences occurred during a 100 day examination period, the infection was considered to have been cured.

Drug doses were calculated as mg base per kg of body weight. Stock solutions of water soluble compounds, at appropriate concentrations, were prepared with distilled water and stored at 8° C for the treatment period. If a compound was water insoluble, a suspension of the requisite amount of drug was prepared daily with 0.3% methylcellulose (in distilled water).

Oral administration of drugs was by gastric intubation with a 14 French catheter. The total volume of fluid administered, drug solution or suspension, and rinse was 14 ml.

Response to treatment was categorized as clearance and cure, clearance and recrudescence, or suppression withouth clearance. The day of clearance was defined as the first of three consecutive days in which the

thick blood films were parasite negative. The day of recrudescence was the first of three consecutive days of positive thick blood films after a period of clearance. Suppression was defined as a transient decrease in the parasite count post-treatment without clearance.

The second objective of this project is to evaluate plasmid DNA vaccines against the blood and sporozoite stages of *P. falciparum* and against the blood stages of *P. vivax* in the Panamanian *Aotus* model. To this end we have evaluated single and multigene DNA vaccines of *both P. falciparum* and *P. vivax* with or without the addition of cytokines. The results of these experiments are detailed in results.

II Results

44. Toxicity of an oral route of administration of GJ-287 (WR282650; BP20546) and GJ-QZ (WR282651; BP20537) in Aotus monkeys.

In order to test the toxicity of an oral route of administration of GJ-287 (WR282650; BP20546) and GJ-QZ (WR282651; BP20537) in an Aotus monkey-model, in June 5, 2001, one (1) male and one (1) female *Aotus lemurinus lemurinus* malaria double cured monkeys weighing (845 and 900 gms), were administered 20 mg/kg of GJ-287 (WR282650; BP20546) or GJ-QZ (WR282651; BP20537) orally for three consecutive days respectively. The animals were monitored daily for signs such as: vomiting, depression, and anorexia and their weight was measured before treatment and twice a week after treatment. Blood was drawn from the femoral vein for CBC and Chemistry determinations such as Creatinine, Blood Urea Nitrogen and GPT before and during treatment and then twice a week for two weeks. No significant changes in body weight nor signs of toxicity were observed in the animals tested. In MN 12959 which received GJ-287 for three days a transient leukocytosis (WBC 49.87) with an absolute lymphocytosis (42.7%) was observed on the third day of treatment. Also during this period an increase in GPT (64.4 UI) was observed in this animal which then return to normal by day 10 post treatment (PT). In contrast, in MN 12960 which received GJ-QZ a leukopenia (WBC 9.71) with an absolute lymphocytosis (47.5%) was observed on the second day of treatment which then return to normal baseline values four days PT. A transient GPT increase on the first day of treatment (54.6 UI) was also present in this monkey.

45. To determine if the co-administration of GJ-287 (WR282650 BN; 20546) and GJ-QZ (WR282651 BP; 20537) alone or in combination with Chloroquine (WR1544 BM;AR20613) against infections of the FVO strain (CQR) of *Plasmodium falciparum* in Aotus monkeys reverse chloroquine resistance.

On June 11, 2001, each of sixteen (16) *Aotus l. lemurinus*, malaria naïve males and females weighing from (735-1046) grams, were divided into eight groups of two monkeys each and inoculated intravenously with 5 x 10^5 FVO strain of *P. falciparum* infected Erythrocytes. Blood films were obtained on the day after inoculation and continued daily for the duration of the experiment. When parasitemia reached 5,000 per ccmm, oral treatment once a day for three days was administered as follows: Group 1. Received Chloroquine (WR1544 BM;AR20613) alone at 10 mg/kg once a day x three days. Group 2. Received GJ-287 (WR282650 BN; 20546) alone at 20 mg/kg once a day x three days. Group 3. Received GJ-QZ (WR282651 BP; 20537) alone at 20 mg/kg once a day x three days. Group 4. Received GJ-287 (WR282650 BN; 20546) at 20 mg/kg plus Chloroquine (WR1544 BM;AR20613) at 10 mg/kg once a day x three days. Group 5. Received GJ-QZ (WR282651 BP; 20537) at 20 mg/kg plus Chloroquine (WR1544 BM;AR20613) at 10 mg/kg once a day x three days. Group 6. Received GJ-287 (WR282650 BN; 20546) at 10 mg/kg plus Chloroquine (WR1544 BM;AR20613) at 10 mg/kg once a day x three days. Group 7. Received GJ-QZ (WR282651 BP; 20537) at 10 mg/kg plus Chloroquine (WR1544 BM;AR20613) at 10 mg/kg once a day x three days. Group 8. Served as Controls (no drug). As shown on table 42, 43 and 44 no effect over parasitemia was observed in the treated groups. One animal from group 4 and another one from group 5 died of malaria related complications on days 20 and 16 PI respectively.

46. Automated blood counts and renal function tests in feral laboratory adapted *Aotus lemurinus lemurinus* from Panamá.

Hematological values have been determined in the past in *Aotus lemurinus lemurinus* from Panamá (Karyotype VIII of IX) (Ma et al, 1978) using a manual system (Porter, 1969). Recently however, on May 14-17, 2001 these values were determined again at this laboratory as part of a Malaria protocol, using an automated hematological counter (MS4, France) and a chemistry analyzer (Reflotron, Merck). Herein, data on hematological and renal chemistry values are presented. Thirty (30) feral laboratory adapted (5-6 month in captivity) (fifteen (15) male and fifteen (15) female) adult *Aotus lemurinus lemurinus* monkeys and weighing between (700-950 grms) were bled from the femoral vein once to determine their blood and renal parameters which included CBC, creatininte and Blood Urea Nitrogen. Results are shown on table 45. No significant difference in CBC counts between male and female monkeys were found except for lymphocytes ($p = 0.05$) and granulocytes ($p = 0.03$) differentials which were significantly different by one-tail student's T-test.

KEY RESEARCH ACCOMPLISHMENTS:

1. No significant changes in body weight nor signs of toxicity were observed in animals that received GJ-287 (WR282650; BP20546) or GJ-QZ (WR282651; BP20537) orally for three days. Only a transient leukocytosis with an absolute lymphocytosis or leukopenia and liver enzyme GPT increase were observed during the experiment.

2. No effect over parasitemia in Aotus infected with choroquine resistant *P. falciparum* FVO, was observed when GJ-287 (WR282650 BN; 20546) or GJ-QZ (WR282651 BP; 20537) were administered orally alone or in combination with Chloroquine (WR1544 BM;AR20613).

3. Automated blood counts and renal function tests in feral laboratory adapted *Aotus lemurinus lemurinus* from Panamá demonstrated no significant difference in CBC counts between male and female monkeys except for lymphocytes ($p = 0.05$) and granulocytes ($p = 0.03$) differentials which were significantly different by one-tail student's T-test.

REPORTABLE OUTCOMES:

I. Manuscripts:

Jones TR, Stroncek DF, Gozalo AS, Obaldia NIII, Andersen EM, Lucas C, Narum DL. Magill AJ, Sim BKL, Hoffman SL. 2001. Anemia in Parasite-and Recombinant Protein-Immunized Aotus Monkeys Infected with *P. falciparum.* Blood. Submitted for publication to Vaccine.

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Ohrt C,....Obaldia N..... Status of Artelinic Acid development. 49th Annual Meeting of The American Society for Tropical Medicine and Hygiene. Westin Galleria & Oaks, Houston, Texas. October 29-November 2, 2000.

Jones TR, Gozalo AS, Obaldia N. et al. Anemia in Aotus Monkeys Infected with *P. falciparum*. 49th Annual Meeting of The American Society for Tropical Medicine and Hygiene. Westin Galleria & Oaks, Houston, Texas. October29-November 2, 2000

Jones TR, Obaldia NIII, Gramzinski RA, Hoffman SL. Repeated Infection of *Aotus* Monkeys with *Plasmodium falciparum* Induces Protection Against Subsequent Challenge with Homologous and Heterologous strains of Parasite. Am J Trop Med Hyg. Presented at the American Society of Tropical Medicine and Hygiene Meeting. Washington DC, November 28-December 2 1999

Obaldia NIII, Jones TR, Gramzinski RA, Charoenvit Y, Kolodny N, Kitov S, Davis HL, Krieg AM, Hoffman SL. Synthetic Oligodeoxynucleotides Containing CpG Motifs Enhance Immunogenicity of a Peptide Malaria Vaccine in Aotus Monkeys. Presented at the American Society of Tropical Medicine and Hygiene Meeting. Washington DC, November 28-December 2, 1999

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CONCLUSIONS:

1. Drugs GJ-287 (WR282650; BP20546) or GJ-QZ (WR282651; BP20537) were non toxic when given orally for three days to Aotus.

2. Drugs GJ-287 (WR282650 BN; 20546) or GJ-QZ (WR282651 BP; 20537) when administered orally alone or in combination with Chloroquine (WR1544 BM;AR20613) had no effect over parastimia in *P falciparum FVO* infected Aotus.

3. No significant differences in CBC counts or Renal function tests except for lymphocytes ($p = 0.05$) and granulocytes ($p = 0.03$) were found among male and female captive laboratory adapted Aotus monkeys.

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TABLE 42

DETAILED ACTIVITY OF GJ-287* (WR282650 BN; 20546) AND GJ-QZ** (WR282651 BP;20537) WITH OR WITHOUT CHLOROQUINE*** (WR1544 BM;AR20613) AGAINST INFECTIONS OF *P. Falciparum* FVO (CQR) IN AOTUS MONKEYS

	RX INITIATED			PARASITEMIA PER ccmm x 10^3								
MONKEY #	DAY P.I	DAY PAT.	MGKG	DAY PRE	DAY OF RX			DAY POST RX				
				RX	1	2	3	1	2	3	4	Days Neg.
13076	8	3	10***	0.13	22.5	3.3	180.7	652.3***	489	174.3	21.6	0
13077	8	4	10***	0.2	17.5	20.4	309	899.9***	808	239.8	73.7	0
13078	8	4	20*	0.01	2.7	2.2	46.5	220.4	365.4	901.4***	178	0
13079	8	4	20*	0.15	8.5	2.9	108.7	418.2***	513	135.5	17.8	0
13080	8	3	20**	0.11	6.9	1.1	196.6	176.6	733.2***	283.1	128.8	0
13081	8	3	20**	0.01	14.4	2.8	211.4	795.7***	922.3	224.9	83.4	0
13082	8	3	20* 10***	0.01	1.34	0.18	13.8	5.9	45.3	217.4	203.1	(***)0
13083	8	3	20* 10***	0.01	26.7	7	108.7	197.8	290.6	1090.2***	707.7	0
13084	8	2	20** 10***	0.01	7.6	0.79	71.2	62.9	307.2	1203.4***	642.1	0
13085	8	3	20** 10***	0.01	3.9	0.68	20.7	1.34	44.2	124.5	196.3	(***)0
13086	8	4	10* 10***	0.18	16.8	1.4	295.9	371.4	1002.6***	625.5	163.9	0
13087	8	3	10* 10***	0.01	16.2	1.8	106.5	416***	238.5	31.2	25.3	0
13088	8	3	10** 10***	0.13	11	0.48	31	96.6	382	1697.2***	862.7	0
13089	8	3	10** 10***	0.01	18.5	20.2	168.7	223.4	549.6***	375	140	0
13090	8	4	CONTROL	0.01	4.6	1.5	152.2	288.4	937.7***		135.5	0
13091	8	3	CONTROL	0.01	3.1	0.42	46.5	85	61.4	671.9***	473.8	0

***=Mefloquine 20 mgkg

(***)=Mefloquine treated 20 mg/kg MN13082 day 15; died day 20 PI. MN13085 day 16; died day 20 PI.

TABLE 43

SUMMARY OF ACTIVITY OF GJ-287* (WR282650 BN; 20546) AND GJ-QZ** (WR282651 BP;20537) WITH OR WITHOUT CHLOROQUINE*** (WR1544 BM;AR20613) AGAINST INFECTIONS OF *P. Falciparum* FVO (CQR) IN AOTUS MONKEYS

Monkey No.	Daily Dose x 3 mg/Kg	Response of parasitemia to Rx			Days from initial Rx to parasite Clearance	Days from final Rx to recrudescence	Notes No of days negative
		None	Suppressed	Cleared			
13076	10***	X					
13077	10***	X					
13078	20*	X					
13079	20*	X					
13080	20**	X					
13081	20**	X					
13082	20*	X					DIED day 20 PI
	10***						
13083	20*	X					
	10***						
13084	20**	X					
	10***						
13085	20**	X					DIED day 16 PI
	10***						
13086	10*	X					
	10***						
13087	10*	X					
	10***						
13088	10**	X					
	10***						
13089	10**	X					
	10***						
13090	Control	X					
13091	Control	X					

TABLE 44

DETAILED PARASITEMIA OF GJ-287* (WR282650 BN; 20546) AND GJ-QZ** (WR282651 BP;20537) WITH OR WITHOUT CHLOROQUINE*** (WR1544 BM;AR20613) AGAINST INFECTIONS OF *P. Falciparum* FVO (CQR) IN AOTUS MONKEYS

Parasitemia x ccmm

PI/DAY	GROUP	06/25/01 3	06/26/01 5	06/27/01 6	06/28/01 7	06/29/01 8	06/30/01 9	07/01/01 10	07/02/01 11	07/03/01 12	07/04/01 13	07/05/01 14	07/06/01 15	07/07/01 16	07/08/01 17	07/09/01 18	07/10/01 19	07/11/01 20	07/12/01 21	07/13/01 22
13076	1	0	0	0.01	130	22500	3320	180750	652320***	489000	174330	21610	590	0.01	0	0	0	0	0	0
13077	1	0	0.01	0.01	200	17510	20410	309000	899960***	808500	239890	73760	2310	0.01	0	0	0	0	0	0
13078	2	0	0.01	0.01	0.01	2770	2220	46500	220460	365420	901470***	178060	34270	1140	0.01	0	0	0	0	0
13079	2	0	0.01	0.01	150	8570	2930	108750	418270***	513000	135590	17880	17880	0.01	0	0	0	0	0	0
13080	3	0	0	0.01	110	6900	1140	196680	176670	733290***	283100	128890	14900	0.01	0	0	0	0	0	0
13081	3	0	0	0.01	0.01	14400	2840	211400	795770***	922310	224990	83440	3870	0.01	0	0	0	0	0	0
13082	4	0	0	0.01	0.01	1340	180	13810	5980	45300	217440	203100	342020	459800***	235500	1050	0.01	DIED		
13083	4	0	0	0.01	0.01	26720	7020	108750	197810	290680	090220***	707750	41720	8940	0.01	0.01	0	0	0	0
13084	5	0	0	0	0.01	7650	790	71250	62920	307290	203470***	642190	32780	4470	0.01	0.01	0	0	0	0
13085	5	0	0	0.01	0.01	3980	680	20700	1340	44290	124500	196300	1015.5***	DIED						
13086	6	0	0.01	0.01	180	16800	1470	295960	371460	002640***	625500	163900	31290	790	0.01	0	0	0	0	0
13087	6	0	0	0.01	0.01	16200	1870	106500	416010***	238580	31290	25330	0.01	0	0	0	0	0	0	0
13088	7	0	0	0.01	130	11000	480	31000	96640	382030	697240***	862710	186.2	15650	730	0.01	0	0	0	0
13089	7	0	0	0.01	0.01	18570	2020	168750	223480	549640***	375000	140060	15000	0.01	0.01	0	0	0	0	0
13090	control	0	0.01	0.01	0.01	4600	1520	152250	288410	937710***	697320	135590	32010	0.01	0.01	0	0	0	0	0
13091	control	0	0	0.01	0.01	3150	420	46500	85000	61410	671950***	473820	40230	0.01	0.01	0	0	0	0	0

***=Treatment with Mefloquine 20 mg/kg

Treatment=

TABLE 45 AUTOMATED HEMATOLOGICAL AND RENAL CHEMISTRY VALUES OF FERAL LABORATORY ADAPTED *Aotus l. lemurinus* MONKEYS FROM PANAMA

	MALES n=15			FEMALES n=15			
	MEAN	**STD**	**RANGE**	**MEAN**	**STD**	**RANGE**	***p***
WBC X 10^3	**24.6**	12.9	11.5-64.0	**30.5**	18.5	13.71-85.5	
LYM %	**27.4**	8.0	14.4-43.7	**31.8**	8.3	22.2-52.5	**0.05*
MON %	**20.1**	4.7	11.8-28.7	**22.5**	6.6	8.9-33.0	
GRA %	**52.6**	11.2	35.2-69.2	**45.6**	12.0	25.6-64.4	**0.03*
RBC X 10^6	**6.1**	0.4	5.42-7.0	**6.1**	0.3	5.64-6.6	
MCV fl	**87.0**	3.1	81-91.8	**86.9**	3.2	80.2-90.6	
HCT %	**52.9**	3.3	47-58.9	**52.7**	2.9	47.7-56.9	
MCH pg	**25.1**	1.5	22.2-27.6	**25.0**	1.5	23-27.7	
RDW	**29.1**	1.3	27.4-31.4	**28.8**	1.4	26.7-30.7	
MCHC g/dl	**8.2**	0.4	7.4-8.9	**8.4**	0.3	7.9-8.8	
Hb g/dl	**15.4**	0.5	14.6-16.4	**15.2**	0.8	13.9-16.9	
PLT x 10^3	**528.9**	122.8	338-771	**513.3**	118.0	343-764	
MPV fl	**11.6**	0.3	11.3-12.2	**11.4**	0.4	10.5-12.0	
Pct %	**0.6**	0.1	0.41-0.9	**0.6**	0.1	0.41-0.9	
PDW	**10.1**	0.8	8.0	**10.2**	0.8	8.9-11.6	
CREA mg/dl	**0.5**	0.0	0.5	**0.5**	0.0	0.5-0.6	
BUN mg/dl	**10.0**	0.0	10.0	**10.9**	2.3	10-17.8	

p= One Tail T-Test